pH-dependent FcRn binding and release assay in a microplate: A new method for screening Fc interactions of mAbs, Bispecifics and Fc engineered antibodies

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Abstract

We present a novel assay based on PAIA microplate technology extending our portfolio of high-throughput developability assays. The *FcRn Binding Assay Kit* (PA-DEV-4FcRn) enables detection of the pH-dependent binding and release of antibodies to FcRn in a simple, fully microplate-based workflow. Antibodies are captured on FcRn-coated beads and analyzed across four wells with different pH values. FcRn is crucial for intracellular antibody recycling and a major determinant of antibody clearance. Efficient recycling requires strong binding at pH 6 (intracellular) and weak binding at pH 7.4 (physiological), ensuring proper FcRn (extracellular) release.

We evaluated different monoclonal antibody (mAb) sample sets and demonstrate that this technology enables rapid and high-throughput FcRn characterization, providing a robust alternative to conventional column- and biosensor-based methods.

Assay principle

Figure 1. Assay workflow on PAIA microplates

The assay is performed in 384-well PAIA*plates* containing dried capture beads [1]. After adding the fluorescent marker and sample [2], the plate is shaken for 60 min [3]. Afterwards, the beads are settled on the bottom, out of the measurement area [4]. Plates are read on a fluorescence plate reader in bottom-read mode [5]. Only the fluorescence in solution is detected, so binders show low fluorescence values.

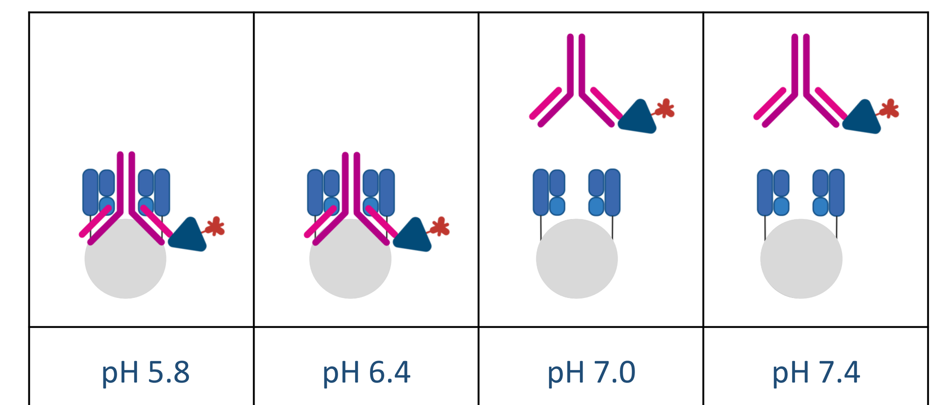


Figure 2. Assay components of the *FcRn Binding Assay Kit* (PA-DEV-4FcRn)

Human FcRn is immobilized on capture beads. A fluorophore-labeled anti-human antibody serves as a marker to monitor analyte (10 µg/mL) release across a pH gradient (5.8–7.4), which can be used to predict antibody clearance.

Materials and methods

Choice of analytes

PA-DEV-4FcRn was evaluated in a proof of principle study using 35 well-characterized clinical-stage antibodies covering diverse properties predictive of clinical success or failure.

In addition, a panel of 43 IgG1 antibodies with published PK data (clearance in hFcRn Tg32 mice[1]) was analyzed. These antibodies were originally selected from a larger set of 334 mAbs to preserve germline diversity and cover the full range of developability characteristics (Figure 3).

Developability assessment

Samples were diluted to 10 µg/mL in DI water. 10 µL of each sample was dispensed into 384-well PAIA*plates*, followed by 30 µL of the fluorescence-labeled marker. Plates were incubated at 1800 rpm for 60 min, centrifuged at 1500 x g, and read in bottom-read mode at 630/665 nm.

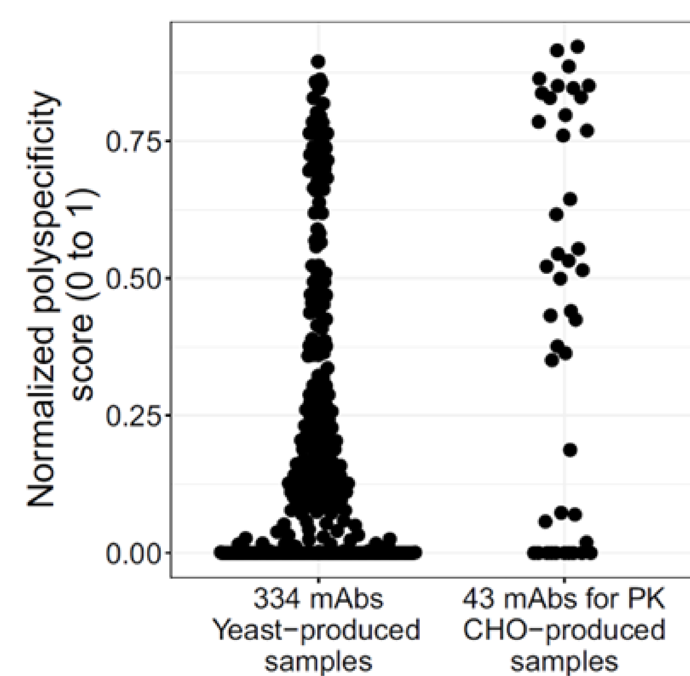


Figure 3. PSR scores of chosen mAbs (from Jain et al., 2024[1]): In the study, the whole range of developability characteristics was shown to be covered for the parental population and the chosen subset of 43 mAbs. Several methods were used, the PSR score is depicted here as an example. The distribution of the 43 chosen mAbs (right) reflects the diversity of the original dataset.

Proof of principle: ustekinumab vs. briakinumab

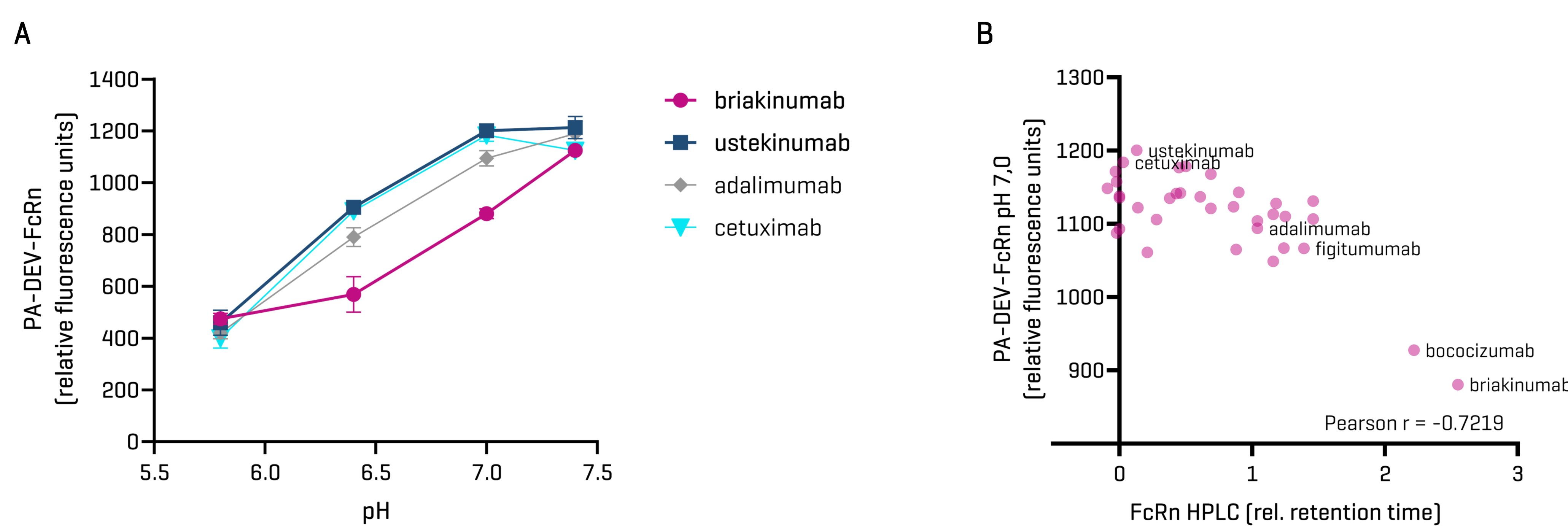


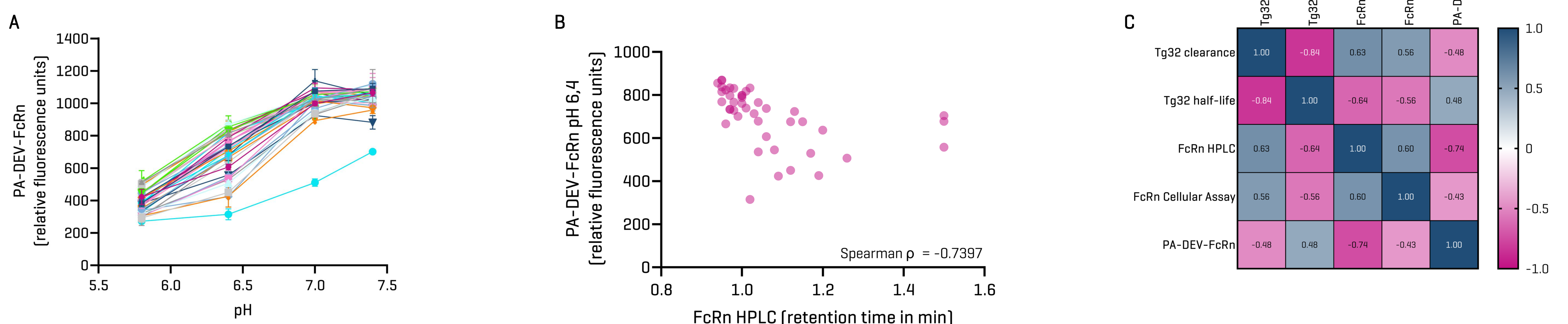
Figure 4. Proof of Principle

Schoch et al.[2] (2015) showed that ustekinumab and briakinumab differ in FcRn interactions and serum half-life. These two antibodies, together with 33 additional mAbs, were included in our proof-of-principle study to validate PA-DEV-4FcRn.

A) pH profiles of four selected clinical-stage mAbs. An increase in fluorescence intensity indicates release from the FcRn-coated beads (n=2).

B) Comparison of PA-DEV-4FcRn results with FcRn HPLC retention time data (Kraft et al., 2020[3]). Fluorescence intensities for 35 mAbs were plotted against HPLC retention times, showing a strong correlation.

Correlation with orthogonal methods



	Tg32 clearance	Tg32 half-life	FcRn HPLC	FcRn Cellular Assay	PA-DEV-FcRn
Tg32 clearance	1.00	-0.84	0.63	0.56	-0.48
Tg32 half-life	-0.84	1.00	-0.64	-0.56	0.48
FcRn HPLC	0.63	-0.64	1.00	0.60	-0.74
FcRn Cellular Assay	0.56	-0.56	0.60	1.00	-0.43
PA-DEV-FcRn	-0.48	0.48	-0.74	-0.43	1.00

Figure 5. Comparison of PA-DEV-4FcRn with published **data**[1]

A) pH profiles of 43 IgG1 antibodies. An increase in fluorescence intensity indicates release from the FcRn-coated beads. B) Comparison of PA-DEV-4FcRn results with FcRn HPLC retention time data (Jain et al., 2024[1]). Fluorescence intensities were plotted against HPLC retention times, showing a strong Spearman correlation. C) Correlation matrix of PA-DEV-4FcRn results with established methods and PK parameters (Tg32 clearance, Tg32 half-life, FcRn HPLC, FcRn cellular assays). PA-DEV-4FcRn showed strong correlations across all methods, with the highest agreement observed with FcRn HPLC. The assay was repeated twice in duplicates, showing very high reproducibility (data not shown).

Literature

1. Jain et al. (2024) "Assessment and incorporation of in vitro correlates to pharmacokinetic outcomes in antibody developability workflows." DOI: 10.1080/19420862.2024.2384104
2. Schoch et al. (2015) "Charge-mediated influence of the antibody variable domain on FcRn-dependent pharmacokinetics." DOI: 10.1073/pnas.1408766112
3. Kraft et al. (2020) "Heparin chromatography as an in vitro predictor for antibody clearance rate through pinocytosis." DOI: 10.1080/19420862.2019.1683432

No-wash developability assays | FcRn assay in a microplate | Hundreds of samples per hour